



# Nitrogen-15 Signals of Anthropogenic Nutrient Loading in Reef Corals

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The  $^{15}\text{N}$  content of tissue from the coral *Porites lobata* was enriched, relative to corals from reference sites, at 5 of 7 Indo-Pacific sewage-affected reefs. Enrichment was as high as 3.7. The  $\delta^{13}\text{C}$  of sewage-affected corals suggests they maintained a high degree of autotrophic nutrition.  $^{15}\text{N}$ -enriched wastewater dissolved inorganic nitrogen (DIN), derived from untreated sewage, is the most likely cause for enrichment in coral tissue  $^{15}\text{N}$ , though changes in coral nutrition, metabolism and zooxanthellae population dynamics are possible additional factors. Isotopic measurements of coral tissue can provide a simple means of detecting wastewater uptake by corals. © 2000 Elsevier Science Ltd. All rights reserved.

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## Introduction

Anthropogenic nitrogen inputs can have profound detrimental effects on ecosystem diversity, composition and function (Vitousek *et al.*, 1997). Coastal marine eutrophication has drastic consequences for coral reefs in particular (Rosenberg, 1985; Tomascik and Sander, 1987; Nixon, 1995). Methods to trace anthropogenic N inputs and to assess their impact on ecosystems are required. The natural abundance ratio of  $^{15}\text{N}/^{14}\text{N}$  has been frequently employed as a tracer of anthropogenic nutrient loading in groundwater, fluvial, lacustrine, es-

tuarine and marine systems (e.g., Sweeney and Kaplan, 1980; Aravena *et al.*, 1993; Bachtar *et al.*, 1996; McClelland *et al.*, 1997).

Wastewater nutrients derived from animal wastes, septic systems and sewage treatment plants are generally enriched in the heavy isotope of nitrogen,  $^{15}\text{N}$ . This is due to nitrogen transformations that typically occur prior to or after discharge of such wastes. These transformations include ammonia volatilization, denitrification of nitrate, and nitrification of ammonia, all of which leave residual dissolved inorganic nitrogen (DIN) with high  $\delta^{15}\text{N}$  (10–22‰; Heaton, 1986; Jordan *et al.*, 1997). Synthetic fertilizer wastes typically have values closer to 0‰ (Heaton, 1986), allowing them to be distinguished from sewage wastes, but nitrogen transformations may obscure differences in source signal (Lindau *et al.*, 1997; Cabana *et al.*, 1999). Sewage particulate organic matter (POM) and sewage sludge can also be identified in freshwater and marine sediments and can be recognized as part of the diet of various organisms (e.g., Sweeney *et al.*, 1980; Sweeney and Kaplan, 1980; Rau *et al.*, 1981; Coakley *et al.*, 1992; Bachtar *et al.*, 1996). This is possible due to its often distinctive, low isotopic signature ( $\sim -1$ – $-7$ ‰; Sweeney and Kaplan, 1980; Van Dover *et al.*, 1992) relative to background organic matter.

Several applications of these tracer techniques have been demonstrated in terrestrial aquatic settings. These include tracing of  $^{15}\text{N}$ -enriched nitrate from septic tank discharges into groundwater aquifers (e.g., Kreitler and Browning, 1983; Aravena *et al.*, 1993; Burg and Heaton, 1998) and tracing wastewater from sugarcane fields into forested wetlands of Louisiana (Lindau *et al.*, 1997). Fish in rivers and lakes affected by sewage wastewater inputs have higher  $\delta^{15}\text{N}$  than those from pristine waters

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(Cabana and Rasmussen, 1996). Sediment organic matter near sewage treatment plants on Lake Ontario has been found to be isotopically distinct relative to surrounding sediments (Coakley *et al.*, 1992; Bachtiar *et al.*, 1996). Woodland plots irrigated with sewage wastewater enriched in  $^{15}\text{N}$  by the treatment process show isotopic enrichment in both soil and foliage (Jordan *et al.*, 1997).

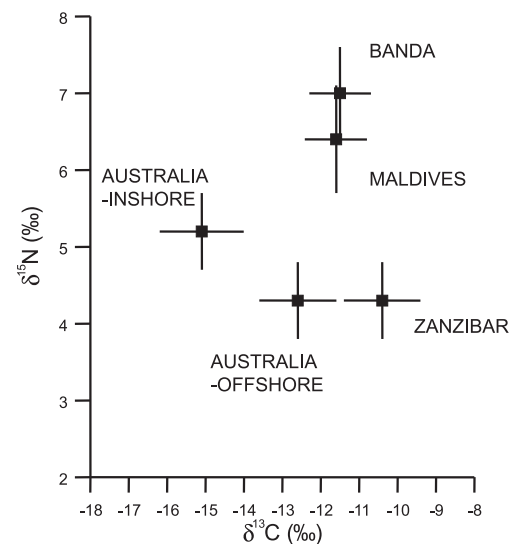
These techniques have also been applied in estuarine and fully marine settings. On Cape Cod, Massachusetts, both primary producers and consumers were found to have heavier  $\delta^{15}\text{N}$  in estuarine settings affected by groundwater DIN of anthropogenic origin. This enrichment is found even at relatively low nitrogen loading rates (McClelland *et al.*, 1997; McClelland and Valiela, 1998). Similar enrichment related to wastewater DIN has been found in sediments (Larsen *et al.*, 1999) and in entire food chains of the Baltic Sea (Hansson *et al.*, 1997). Low  $\delta^{15}\text{N}$  values, due to particulate sewage inputs, have also been observed in marine systems. Sedimentary organic matter offshore southern California records particulate sewage input (Sweeney and Kaplan, 1980; Sweeney *et al.*, 1980). Rau *et al.* (1981) have recorded particulate sewage uptake into estuarine food webs. In these cases the POM or biota are depleted in  $^{15}\text{N}$ , since sewage particulate organic matter is generally depleted in  $^{15}\text{N}$  relative to marine organic matter (Owens, 1987). Uptake of isotopically light particulate sewage sludge has also been recorded in deep-sea food webs (Van Dover *et al.*, 1992).

$^{15}\text{N}$  natural abundance tracer techniques have also been applied in tropical reef environments. Stomatopods (decapod crustaceans) on sewage-affected Indonesian reef flats are enriched in  $^{15}\text{N}$  (Risk and Erdmann, 2000). This may be due to consumption of faecal matter enriched in  $^{15}\text{N}$  relative to marine organic matter or due to utilization of  $^{15}\text{N}$ -enriched wastewater at the base of the food chain. Faecal matter would be enriched relative to reef organic matter if nitrogen fixation was a predominant source of nitrogen in the reef settings studied (Risk and Erdmann, 2000).  $^{15}\text{N}$  enrichments have been found in mangrove leaves, and reef molluscs, fishes and corals, in settings exposed to anthropogenic nutrient pollution (Cabana, 1997; Risk and Heikoop, 1997; Mendes *et al.*, 1997; Fry *et al.*, 1999). At least in the case of primary producers (mangroves) or organisms that rely on symbiotic algae (corals), much of this enrichment could be attributed to assimilation of wastewater DIN.

This paper deals with the effect of anthropogenic nutrient enrichment on the  $\delta^{15}\text{N}$  of reef coral tissue. Two factors have been identified which affect the  $\delta^{15}\text{N}$  of coral tissue in oligotrophic settings: the  $\delta^{15}\text{N}$  of source nutrient and nitrogen isotopic fractionation associated with light availability/photosynthesis (Keegan and DeNiro, 1988; Muscatine and Kaplan, 1994; Yamamuro *et al.*, 1995; Heikoop, 1997; Heikoop *et al.*, 1998; Sammarco *et al.*, 1999; Heikoop *et al.*, in press).

Variability in source nutrient isotopic composition can cause a wide range in the  $\delta^{15}\text{N}$  of shallow water corals in which fractionation of DIN is minimized (Fig. 1). For example, corals from reefs which receive abundant inputs of terrestrial nitrogen, upwelled nitrogen (from non-denitrifying zones) and from nitrogen fixation have lower  $\delta^{15}\text{N}$  (e.g., Great Barrier Reef; Fig. 1; Keegan and DeNiro, 1981; Yamamuro *et al.*, 1995; Heikoop, 1997; Sammarco *et al.*, 1999; Heikoop *et al.*, in press).

Under conditions in which abundant zooxanthellar photosynthesis occurs, all of the DIN entering the coral internal DIN pool will be utilized and only fractionation associated with initial uptake will be expressed (Heikoop *et al.*, 1998). Corals collected under lower light conditions (with lower rates of photosynthesis) have lower  $\delta^{15}\text{N}$  values due to increased expression of fractionation of DIN during uptake and assimilation by zooxanthellae (Heikoop *et al.*, 1998). Fractionation related to excretion of isotopically depleted nitrogen is probably not expressed in corals due to efficient recycling of nitrogen between host and symbionts (Eustice *et al.*, 1995; Heikoop *et al.*, 1998). Therefore, typical  $^{15}\text{N}$  trophic level enrichments ( $\sim 3.5\text{‰}$ ; DeNiro and Epstein, 1981; Minagawa and Wada, 1984) are not observed. Shallow water corals, therefore, could prove to be good



**Fig. 1**  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of shallow (< 5 m) *P. lobata* tissue from various Indo-Pacific reefs. Squares represent mean values. Range bars represent one standard deviation on either side of the mean. Data presented here is a subset of data presented in Heikoop (1997) and Heikoop *et al.* (in press). Zanzibar and Maldives data (much of which was included as part of present study) include sewage-affected and reference corals, but sewage-related differences at these sites (Table 1) are much smaller than the inter-reef differences displayed in this figure. Australian coral data are for inshore corals and mid-shelf to outer-shelf corals (termed offshore corals here). See Risk *et al.* (1994b) and Sammarco *et al.* (1999) for an explanation of cross-shelf trends in coral tissue  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  for corals of the central Great Barrier Reef. See Heikoop (1997) and Heikoop *et al.* (in press) for explanations of the isotopic spread shown here.

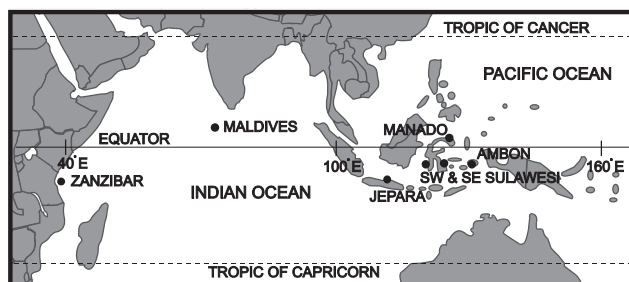


Fig. 2 Map showing general locations of study areas.

biomonitors of ambient DIN isotopic values that are sensitive to sewage nutrient input.

Under conditions of nutrient enrichment, the  $\delta^{15}\text{N}$  of coral tissue may be altered by the isotopic composition of anthropogenic dissolved nutrients or by light-related fractionation. In addition, because of the mixotrophic nature of corals, under nutrient-enriched conditions corals may switch from an autotrophic to a heterotrophic mode of nutrition. Since heterotrophic food sources (e.g., zooplankton) are isotopically distinct relative to DIN (e.g., Muscatine and Kaplan, 1994), this could also affect the  $\delta^{15}\text{N}$  of coral tissue. Heterotrophic food sources could include particulate faecal wastes with low  $\delta^{15}\text{N}$  or zooplankton with higher  $\delta^{15}\text{N}$ . Carbon isotopes are used here to distinguish between autotrophic and heterotrophic modes of coral nutrition (cf. Risk *et al.*, 1994b).

Here we compare the  $\delta^{15}\text{N}$  of corals from sewage-affected reefs with those from reference reefs from settings in Indonesia, Zanzibar and Maldives (Fig. 2).  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements are utilized to discriminate between the various isotopic effects which contribute to the observed  $\delta^{15}\text{N}$  signal of anthropogenic nutrient loading. These isotopic techniques are used not only to trace sewage transport on reefs, but also to show the actual uptake and assimilation of sewage nutrients by corals.

### Sample Collection and Analysis

Five or more *Porites lobata* corals were collected from each reef at depths of less than 5 m. Samples were prepared by the method described in Heikoop *et al.* (1998). An area of approximately 50 cm<sup>2</sup> was chiseled from the upper growth surface of each colony. Excess skeletal material was removed with a diamond saw, prior to decalcification in dilute (10%) HCl. Such acid treatment has been shown to have no effect on coral tissue  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  relative to waterpiked samples (Heikoop *et al.*, 1998). Coral tissue was then rinsed in distilled water and freeze-dried prior to analysis. Approximately 15 mg of tissue was employed for each  $\delta^{15}\text{N}$  analysis, and 5 mg of tissue was employed for each  $\delta^{13}\text{C}$  determination. All samples represent combined host tissue and zooxanthellae.

Samples were combusted for 2 h at 550°C one day prior to analysis. Gases were cryogenically purified prior

to measurement on a VG SIRA mass spectrometer. Typical precision based on replicates of coral tissue samples and in house organic standards is  $<0.1\text{‰}$  (1  $\sigma$ ).

Sampling locations are shown in Fig. 2. All affected reefs show evidence of nutrient enrichment, including one or more of the following: nutrient measurements and chlorophyll A analyses, ecosystem responses including coral cover and diversity, mortality index, urchin and macroalgae abundance, bioerosion and coral associate density. For details on sites and site selections refer to individual theses (cited in Fig. 3 caption) and also Risk *et al.* (1994a) and Edinger *et al.* (1998). The affected reefs receive nutrient loading predominantly

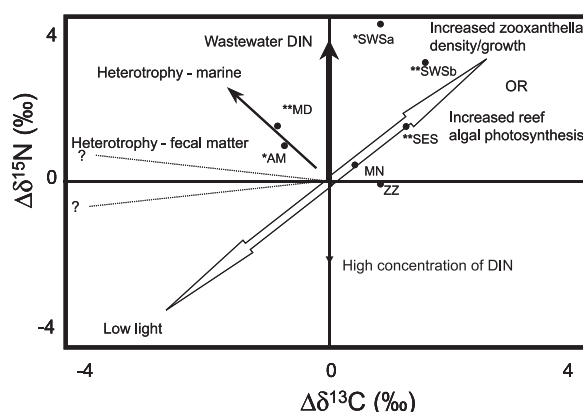


Fig. 3  $\Delta\delta^{15}\text{N}$  versus  $\Delta\delta^{13}\text{C}$  for various Indo-Pacific reefs. All values represent the average isotopic difference between corals (*P. lobata*) at affected reefs versus reference reefs in ‰ (mean  $\delta^{15}\text{N}$  of affected corals minus mean  $\delta^{15}\text{N}$  of reference corals for each site). Sites with significant differences in  $\delta^{15}\text{N}$  relative to reference reefs are identified by an asterisk. Double asterisks mark sites with significant differences in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ . Uptake of wastewater DIN will cause enrichment in coral tissue  $^{15}\text{N}$ . Heterotrophic feeding on marine POM will also tend to raise coral tissue  $\delta^{15}\text{N}$  but lower coral tissue  $\delta^{13}\text{C}$ . Heterotrophic feeding on faecal matter should lower  $\delta^{13}\text{C}$  relative to reference corals. The nitrogen isotopic effect of feeding on faecal matter will depend on the average background N isotopic composition of the reef environment (as indicated by dashed lines and question marks; see text). Reduced irradiance will result in small isotopic depletions in both carbon and nitrogen isotopic coral tissue signatures, while increases in zooxanthellar density could have the opposite effect (see text). Similarly, increased concentration of DIN might also lead to depletion in coral tissue  $^{15}\text{N}$  (see text). Higher  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of coral tissue could result from the effect of increased reef algal productivity on the isotopic composition of reef DIC and DIN pools. The net effect of sewage/wastewater on coral tissue  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  will depend on the relative magnitudes of all these various influences. Corals from most sewage-affected reefs are enriched in  $^{15}\text{N}$ . Clear patterns in  $\delta^{13}\text{C}$  between reference and affected reefs are not seen, but most corals have  $\delta^{13}\text{C}$  values consistent with a largely autotrophic diet (Table 1). This suggests that most of the enrichment in  $^{15}\text{N}$  is due to source effects ( $^{15}\text{N}$ -enriched sewage-derived wastewater). In general,  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$  values of less than 0.5 per mille are unlikely to be significant, due to analytical uncertainty, natural intra-site variation and difficulties in choosing appropriate reference reefs. Site key and author(s) of original study: AM: Ambon (Limmon, 1996); MD: Maldives (Risk *et al.*, 1994a; note additional corals were included in the present data set); MN: Manado (Lazier, 1997); SWSa: SW Sulawesi (Lazier, 1997); SWSb: SW Sulawesi (Jompa, 1996); SES: SE Sulawesi (Lazier, 1997); ZZ: Zanzibar (Dunn, 1995).

from untreated sewage (human and animal) discharges. In south-west Sulawesi, Kayangan, a sewage-affected site offshore the major city Ujung Pandang, was used in two separate studies and compared to different reference reefs (see Table 1).

Fig. 1 illustrates the possibility of obtaining statistically significant differences in  $\delta^{15}\text{N}$  even when comparing relatively unaffected reefs. In eight out of 10 cases, differences in mean  $\delta^{15}\text{N}$  between reefs are significant (Heikoop *et al.*, in press). These differences are driven by variability in factors such as the  $\delta^{15}\text{N}$  of natural DIN sources, as opposed to anthropogenic inputs. Because large background differences exist in the  $\delta^{15}\text{N}$  of corals from reefs not receiving abundant inputs of sewage it is important to attempt to choose reference sites that would have as similar nutrient inputs as possible, in the absence of anthropogenic nutrient loading. We attempted to do this where practical, but in some cases relatively clean reference sites were only available at offshore islands (south-west Sulawesi and Manado). In these cases we sampled reefs offshore the main pollution plume, but not so far offshore that they were in a totally different nutrient environment (e.g., shelf edge upwelling zone). Few of the reference sites are truly 'clean' but all have many fewer inhabitants than affected sites chosen near major population centres.

Data are presented as  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$  in units of ‰, representing the average  $\delta^{15}\text{N}$  (or  $\delta^{13}\text{C}$ ) of sewage-affected corals minus the average  $\delta^{15}\text{N}$  (or  $\delta^{13}\text{C}$ ) of corals from reference reefs. When data were normally distributed (as determined by normal probability plots) and showed no homogeneity of variance problems (as determined by an *F*-test) *t*-tests with pooled standard

deviations (assuming equal variances) were applied. For data that were not normally distributed, logarithmic and square root transformations were applied to see if these made the distributions more nearly normal. If the transformations were not of value, non-parametric Mann–Whitney tests were applied. This was the case for south-west Sulawesi (b) reefs, where transformations failed to improve the normality of the reference  $\delta^{15}\text{N}$  and the affected  $\delta^{13}\text{C}$  data. Pooled  $\delta^{15}\text{N}$  data from the two affected Ambon sites were not normally distributed, so a logarithmic transformation ( $\log_{10}$ ) was applied. This transformation was successful in removing the normality problem, but homogeneity of variance problems was detected. *t*-tests without pooling standard deviations (i.e., we did not assume equal variance) were therefore applied. Data from the two Ambon affected sites were pooled, as were data from the two reference south-west Sulawesi (b) sites, based on the fact that in each case data were statistically indistinguishable for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (*t*-test for Ambon data and Mann–Whitney test for south-west Sulawesi (b) data).

## Results and Discussion

Summary data are shown in Table 1 and Fig. 3. In 5 out of 7 cases, coral tissue  $\delta^{15}\text{N}$  was significantly higher at the polluted site than the reference site.  $\Delta\delta^{15}\text{N}$  ranged from 0.9‰ to 3.7‰ at these five sites.  $\Delta\delta^{15}\text{N}$  averaged  $2.0 \pm 1.2\text{‰}$  ( $1\sigma$ ) for the five sites where significant differences were observed. Mean  $\delta^{15}\text{N}$  was significantly different ( $p < 0.05$ ) at Kayangan over two separate study periods. The reason for this is unknown, though it has been proposed that temporal variability in

**TABLE 1**  
Statistical summary of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  of corals from sewage affected and reference sites.

Region	Site	Status	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		<i>N</i>	$\Delta\delta^{15}\text{N}^a$	Sig.	$\Delta\delta^{13}\text{C}^a$	Sig.
			<i>x</i>	S.D.	<i>x</i>	S.D.					
Maldives	Male	Affected	7.2	0.3	-12.1	0.8	6	1.1	$p < 0.001$	-0.8	$p < 0.05$
	Addu	Reference	6.1	0.5	-11.3	0.6	10				
Zanzibar	Grave	Affected	4.2	0.3	-10.0	0.8	10	0	NS <sup>b</sup>	0.7	NS
	Chumbe	Reference	4.2	0.5	-10.7	1.0	9				
Jepara <sup>c</sup>	P. Panjang	Affected	7.1	1.9	-12.5	0.9	20				
SW Sulawesi a	Kayangan	Affected	9.3	0.9	-11.2	1.3	5	3.7	$p < 0.0001$	0.7	NS
	Bone Tambung	Reference	5.6	0.6	-11.9	1.4	5				
SW Sulawesi b	Kayangan	Affected	8.0	0.6	-12.7	1.7	6	2.9	$p < 0.001$	1.4	$p < 0.05$
	B. Lompo <sup>d</sup>	Reference	5.0	1.3	-14.1	1.5	9				
	Samalona <sup>d</sup>	Reference	5.3	0.9	-14.1	2.4	8				
SE Sulawesi	Kendari	Affected	7.3	0.3	-13.1	1.1	5	1.3	$p < 0.001$	1.1	$p < 0.05$
	P. Mapara	Reference	6.0	0.5	-14.2	0.3	5				
Manado	Manado	Affected	6.8	0.7	-10.9	0.9	6	0.5	NS	0.3	NS
	N. Bunaken	Reference	6.3	0.9	-11.2	1.2	5				
Ambon	Wayame <sup>e</sup>	Affected	8.4	1.2	-13.5	1.1	8	0.9	$p < 0.05$	-0.6	NS
	Wailiha <sup>e</sup>	Affected	8.8	1.5	-12.8	0.9	9				
	Hila	Reference	7.7	0.4	-12.5	0.8	9,11 <sup>f</sup>				

<sup>a</sup>  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  of affected site – reference site.

<sup>b</sup> Not significant,  $p > 0.05$ .

<sup>c</sup> No comparable nearshore reference site was available.

<sup>d</sup> Data from these two reference sites were pooled in calculating  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$ .

<sup>e</sup> Data from these two affected sites were pooled in calculating  $\Delta\delta^{15}\text{N}$  and  $\Delta\delta^{13}\text{C}$ .

<sup>f</sup>  $N=9$  for nitrogen, and 11 for carbon.

proportions of various nutrient inputs could affect coral tissue  $\delta^{15}\text{N}$  (Heikoop *et al.*, in press).  $\Delta\delta^{13}\text{C}$  values were more variable: in 4 out of 7 cases there was no significant difference between affected and reference reefs. Corals from affected reefs at Maldives had significantly lower  $\delta^{13}\text{C}$  than reference reefs, while corals affected by pollution at south-east Sulawesi and south-west Sulawesi (b) were significantly enriched in  $^{13}\text{C}$ . In the case of south-east Sulawesi data this was only true if standard deviations were pooled when calculating the test statistic. The *F*-test for this data set gave a value of  $p = 0.05$ , so the data may or may not have equal variance. If standard deviations were not pooled, no significant differences could be detected in  $\delta^{13}\text{C}$  between south-east Sulawesi reference and affected sites. Reference corals from most sites have  $\delta^{13}\text{C}$  values suggestive of a primarily autotrophic mode of nutrition (Table 1; autotrophic corals typically have  $\delta^{13}\text{C}$  of  $-14$  to  $-10\text{‰}$ ; e.g., Muscatine *et al.*, 1989; Risk *et al.*, 1994b; Heikoop, 1997; Heikoop *et al.*, in press). South-east Sulawesi and south-west Sulawesi (b) reference corals, however, have  $\delta^{13}\text{C}$  values suggestive of perhaps a small degree of heterotrophy, probably related to consumption of terrestrial organic matter (cf. Risk *et al.*, 1994b). No significant correlation exists between  $\Delta\delta^{13}\text{C}$  and  $\Delta\delta^{15}\text{N}$  ( $r = 0.358$ ,  $p = 0.43$ ).

Coral tissue  $\delta^{15}\text{N}$  was significantly higher at affected sites in approximately 70% of the cases, suggesting that  $\delta^{15}\text{N}$  of coral tissue is a good indicator of both sewage pollution on reefs and uptake of sewage nutrients into corals. In no instance did we get a false positive result in which  $\delta^{15}\text{N}$  was significantly higher at the reference site. Several factors, illustrated in Fig. 3, could contribute to the observed signal. To distinguish between these factors it is first necessary to address the trophic status of these corals.

#### *Trophic status of sewage-affected corals*

The high  $\delta^{13}\text{C}$  values of the sewage-affected corals suggest a primarily autotrophic mode of nutrition. Under the somewhat lower light conditions associated with nutrient enriched sites, however, it is likely that corals experience some increase in heterotrophic feeding, either on faecal matter or zooplankton, which would tend to lower  $\delta^{13}\text{C}$  (Fig. 3; Muscatine *et al.*, 1989; Risk *et al.*, 1994b). Increased expression of zooxanthellar fractionation of dissolved inorganic carbon (DIC) under lower light conditions would also tend to cause depletion in coral tissue  $^{13}\text{C}$  (Fig. 3; see depletion-diffusion isotopic model of Muscatine *et al.*, 1989). In 5 out of 7 cases (two significant at  $p < 0.05$ ), however,  $\delta^{13}\text{C}$  was higher at affected sites than reference sites, not supporting increased heterotrophy in sewage-affected corals. Isotopic evidence for any increases in heterotrophic feeding is masked by other factors.

Zooxanthellar fractionation of DIC may be reduced due to dramatic increases in zooxanthellae population densities under conditions of nutrient enrichment

(cf. Stimson and Kinzie III, 1991; Jokiel *et al.*, 1994; Steven and Broadbent, 1997; the corals at our sewage-affected sites were generally more darkly pigmented than reference corals). While photosynthesis by individual zooxanthellae will be somewhat reduced under lower light conditions, total carbon demand may remain high due to the large number of zooxanthellae. This high zooxanthellar demand for carbon from the coral internal DIC pool would result in reduced fractionation, perhaps even relative to corals from reference reefs with somewhat higher light levels. Other factors might contribute to enrichment in coral tissue  $\delta^{13}\text{C}$  (Fig. 3) and mask any isotopic evidence for small increases in heterotrophy. Increased reef macroalgae and phytoplankton productivity, for instance, will preferentially remove  $^{12}\text{C}$  from the reef DIC pool (see for example, review in Goericke *et al.*, 1994), leaving the residual pool enriched in  $^{13}\text{C}$ . This effect occurs in general for all surface waters and is likely to be small in magnitude. The balance of the various effects shown in Fig. 3 will determine if sewage-affected corals are enriched or depleted in  $^{13}\text{C}$  relative to corals from reference reefs. Even with small opposing isotope effects, strongly heterotrophic corals would be expected to approach the  $\delta^{13}\text{C}$  of heterotrophic food source (i.e., approximately  $-20\text{‰}$  based on measurements of faecal matter and reef POM at Jepara; see below). It appears that while there is possibly some small increase in heterotrophy (masked by opposing isotopic effects), sewage-affected *Porites* in shallow water are still able to maintain a significant degree of autotrophy, presumably as a result of increased zooxanthellae populations related to increased levels of DIN.

#### *Origin of the $\delta^{15}\text{N}$ sewage signal*

The  $^{15}\text{N}$ -enriched signal seen in sewage-affected corals probably results from autotrophic assimilation of  $^{15}\text{N}$ -enriched DIN and to a lesser extent, from a small increase in heterotrophic feeding on  $^{15}\text{N}$ -enriched food (e.g., zooplankton; Fig. 3). Zooplankton and reef particulate organic matter may have higher  $\delta^{15}\text{N}$  than usual at sewage-polluted sites if  $^{15}\text{N}$ -enriched wastewater is utilized by phytoplankton at the base of the food chain (cf. Hansson *et al.*, 1997).

Heterotrophic feeding on faecal dissolved organic matter (DOM) or POM could also raise coral tissue  $\delta^{15}\text{N}$  if it is enriched relative to marine organic matter. Particulate sewage is generally depleted in  $^{15}\text{N}$  relative to marine organic matter (e.g., Sweeney and Kaplan, 1980; Rau *et al.*, 1981), but Risk and Erdmann (2000) point out that since nitrogen fixation predominates on many reefs (e.g., Wilkinson *et al.*, 1984), reef organic matter may have lower  $\delta^{15}\text{N}$  than sewage.

Samples of POM, dominated by untreated faecal matter, collected from the river mouth at Jepara, Indonesia had  $\delta^{13}\text{C}$  of  $-21.8\text{‰}$  and  $\delta^{15}\text{N}$  of  $7.9\text{‰}$ . The faecal material was slightly enriched in  $^{15}\text{N}$  relative to POM collected near the island Pulau Panjang at the

head of Jepara Bay (samples collected at 3:30 PM – 20.9‰ and 5.9‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively; samples collected at 7:00 PM when demersal zooplankton were coming to the surface – 20.6‰ and 7.4‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  respectively). Since both marine zooplankton/POM and faecal material have low  $\delta^{13}\text{C}$ , while sewage-affected corals are not depleted in  $^{13}\text{C}$  relative to reference corals, these sources are unlikely to be major portions of the coral diet. Any untreated faecal matter that is consumed by corals, however, could contribute to enrichment in coral tissue  $^{15}\text{N}$  since it has relatively high  $\delta^{15}\text{N}$  (and is enriched relative to reef POM), if the Jepara setting is representative (Fig. 3).

Given the fact that sewage-affected *P. lobata* appear to maintain a relatively high degree of autotrophic nutrition, DIN will be a quantitatively important part of coral diet (cf. Muscatine and Kaplan, 1994; Yamamuro *et al.*, 1995; Eustice *et al.*, 1995; Heikoop, 1997; Heikoop *et al.*, 1998; Sammarco *et al.*, 1999). Since sewage-derived wastewater DIN is typically enriched in  $^{15}\text{N}$  (10–22‰; Heaton, 1986) this source factor may be responsible for much of the observed enrichment in coral tissue  $^{15}\text{N}$  (Fig. 3). Residual ammonium, enriched in  $^{15}\text{N}$  following nitrification, may be particularly important in this regard, as ammonium appears to be a preferred DIN source of many coral/zooxanthellae symbiotic associations (e.g., Muscatine and D'Elia, 1978; Burris, 1983). Increased reef algal productivity under conditions of eutrophication may further raise the  $\delta^{15}\text{N}$  of reef DIN due to preferential algal uptake of  $^{15}\text{NO}_3^-$ . Other autotrophic marine organisms which have been shown to be enriched in  $^{15}\text{N}$  due to utilization of sewage-derived wastewater include mangroves (Fry *et al.*, 1999), phytoplankton, seagrass and macroalgae (Hansson *et al.*, 1997; McClelland *et al.*, 1997; McClelland and Valiela, 1998).

Low light/photosynthesis typically causes depletion in coral tissue  $\delta^{15}\text{N}$  (Fig. 3; Muscatine and Kaplan, 1994; Heikoop *et al.*, 1998). These fractionations, however, are relatively small (~2‰ over the depth range 0–30 m at Discovery Bay, Jamaica; Heikoop *et al.*, 1998). As in the case of  $\delta^{13}\text{C}$ , increased zooxanthellar densities probably reduce this fractionation. Even under low light conditions, if all the DIN entering the coral internal DIN pool is assimilated by the zooxanthellae, then only fractionation associated with the initial uptake of DIN into the coral tissue will be expressed (Heikoop *et al.*, 1998). If this fractionation is very small or constant, then corals could prove to be good tracers of reef DIN.

Similarly, increased concentration of DIN has been hypothesized to increase expression of zooxanthellar fractionation against  $^{15}\text{N}$  (Muscatine and Kaplan, 1994; Heikoop *et al.*, 1998). Because sewage-affected corals are mostly enriched in  $^{15}\text{N}$ , as in the case of light-related fractionations the concentration-related fractionation is either reduced by increased numbers of zooxanthellae consuming DIN from the internal DIN pool, or is overwhelmed by the source signal (i.e., the high  $\delta^{15}\text{N}$  of

wastewater DIN). If for both light and concentration effects, fractionation is reduced by increased zooxanthellar densities, then coral tissue  $\delta^{15}\text{N}$  could prove to be a relatively quantitative tracer of wastewater DIN. If these fractionations are simply overwhelmed by the DIN source signal, then only qualitative tracing is possible. Comparing coral  $\delta^{15}\text{N}$  to  $\delta^{15}\text{N}$  of primary producers such as macroalgae could help assess the degree to which coral tissue  $\delta^{15}\text{N}$  is a quantitative tracer of DIN.

#### *Fidelity of the $\delta^{15}\text{N}$ sewage signal*

Our results indicate that  $\delta^{15}\text{N}$  of coral tissue can be used as an indicator of anthropogenic N loading if caution is used in selecting reference sites. It is important in choosing reference and affected sites to choose sites that would have nitrogen inputs that would be as similar as possible in the absence of anthropogenic nutrient input (Heikoop *et al.*, in press). On the Great Barrier Reef (GBR) of Australia, inshore corals are enriched in  $^{15}\text{N}$  relative to midshelf corals because terrestrial nitrogen, which predominates inshore, has higher  $\delta^{15}\text{N}$  than nitrogen derived from nitrogen fixation, which dominates on midshelf reefs (Sammarco *et al.*, 1999). Natural onshore to offshore variation could confound attempts to look at  $\delta^{15}\text{N}$  sewage signals along nutrient gradients, as opposed to applying the reference to affected site comparisons we have utilized here. Onshore to offshore variation could be a factor in our analysis of south-west Sulawesi reefs where reference sites are from islands 10–15 km offshore. Nearshore south-west Sulawesi corals might be enriched relative to offshore corals even in the absence of sewage pollution if natural terrestrial nutrient inputs were enriched in  $^{15}\text{N}$  relative to nitrogen inputs to reefs further offshore. The inshore sewage-affected corals are highly enriched in  $^{15}\text{N}$ , however, compared to inshore Australia corals receiving terrestrial nitrogen inputs which have an average value of ~4‰ (Owens, 1987). This suggests an additional input of highly  $^{15}\text{N}$ -enriched sewage-derived DIN. Similar inshore–offshore patterns are seen in south-west Sulawesi stomatopods, with samples collected near the major city of Ujung Pandang showing large enrichments in  $^{15}\text{N}$  (Risk and Erdmann, 2000). Corals and stomatopods from lightly inhabited versus uninhabited south-west Sulawesi islands can be distinguished based on  $\delta^{15}\text{N}$ , with inhabited islands showing higher values (Jompa, 1996; Risk and Erdmann, 2000).

In a cross-shelf transect of south-west Sulawesi corals, Jompa (1996) found that the sewage signal was evident to nearly 10 km offshore from the major city Ujung Pandang, though it decreased exponentially with distance from shore. Comparable trends were seen for stomatopods (Risk and Erdmann, 2000). Similarly, corals recorded the sewage signal from Jepara up to several kilometers offshore (Dunn, 1995).

In the case of Zanzibar and Manado, no clear  $\delta^{15}\text{N}$  sewage signal was found. The sewage-affected reef offshore Zanzibar was about 1 km away from the city. It is



possible that a combination of mixing of wastewater and background DIN in prevalent south to north currents may have diluted the signal at this site. Zanzibar is also a major shipping port. Therefore, it is also possible that DIN derived from mineralization of isotopically depleted petroleum (0.7–8.3‰; Faure (1986, p. 520)) may have lowered the  $\delta^{15}\text{N}$  of wastewater DIN. Manado reference corals were collected from an offshore island (Bunaken) which may receive localized sewage inputs from the many losmen (boarding houses) that have been built on the island, as well as wastewater nutrient from the mainland city (Manado) transported by residual currents (Lazier, 1997).

#### Significance to coral metabolism

Sewage pollution is one of the greatest anthropogenic threats to reefs (Pastorok and Bilyard, 1985), particularly in south-east Asia (Wilkinson *et al.*, 1994). Simple techniques are needed that can be used to trace sewage input to reefs and that can help assess its impact on coral health and metabolism. While measurement of  $\delta^{15}\text{N}$  of primary producers such as reef macroalgae may be simpler to detect sewage pollution on reefs, one advantage of  $\delta^{15}\text{N}$  measurements of coral tissue is that it directly demonstrates that corals are consuming wastewater nutrients. Excess nutrient uptake can lead to increases in zooxanthellar density and ultimately decreases in translocation of carbon and nitrogen to the coral host, as the coral loses control of the symbiotic relationship (Stimson and Kinzie III, 1991; Jokiel *et al.*, 1994). The fact that the  $\delta^{13}\text{C}$  of most sewage-affected corals was not lower under conditions of nutrient enrichment, despite expected decreases due to lower light levels (and perhaps increased heterotrophy), suggests that this change in the symbiotic relationship may be occurring. Since the corals do not show evidence for strong reliance on heterotrophy, translocation has not been reduced to the point that the coral hosts must rely largely on heterotrophic food sources.

While coral cover and diversity are often greatly reduced on eutrophied reefs, individual corals may have growth (linear extension) rates as great as corals from cleaner environments. This has been demonstrated on several of the reefs studied here (Risk *et al.*, 1994a; Edinger *et al.*, 1998; Edinger *et al.*, in press). The isotopic data presented here offer clues to the nutrition and metabolism of sewage-affected corals that might help explain such growth rate patterns.

## Conclusions

Enrichment in  $^{15}\text{N}$  of *P. lobata* coral tissue appears to be a promising signal of sewage pollution on coral reefs. The  $\delta^{13}\text{C}$  of sewage-affected corals suggests that the corals have a largely autotrophic mode of nutrition. The most likely cause of coral tissue enrichment in  $^{15}\text{N}$ , therefore, is the uptake of  $^{15}\text{N}$ -enriched wastewater DIN derived from sewage. Sewage-derived wastewater is of-

ten highly enriched in the heavy isotope of nitrogen due to nitrogen transformations.

This technique can complement other techniques for detecting sewage input to marine environments. For particulate matter, these include measurement of faecal steroids (e.g., coprostanol), synthetic organic compounds (e.g., long-chain linear alkyl benzenes), inorganic constituents such as trace elements, faecal bacteria and stable isotope measurements of POM (see review in Vivian, 1986). For dissolved nutrients, direct nutrient measurements and Chlorophyll A analyses could also be utilized. The primary advantage of the technique presented here is that, while it may not prove to be a conservative tracer, it directly demonstrates assimilation of wastewater DIN by the corals, which can potentially have profound effects on coral metabolism.

It would be interesting to repeat this study utilizing corals that are primarily heterotrophic feeders under oligotrophic conditions. Such corals may record particulate sewage input to reefs, while autotrophic corals like *Porites* record inputs of sewage-derived wastewater DIN. If particulate sewage was an important part of coral diet, then sulphur isotopes may be an excellent additional indicator of consumption of sewage particles because of the large isotopic separation relative to marine organic matter. This technique has been applied, for instance, to study the importance of sewage to the diet of blue mussels offshore Massachusetts (Tucker *et al.*, 1999).

Further work will be needed to fully understand the nature of the  $\delta^{15}\text{N}$  sewage signal in corals. Isotopic characterization of separated zooxanthellae and host tissue will help sort out the relative contribution of source versus metabolic effects. Isotopic measurements of all sources of nitrogen to corals, including DIN, should be undertaken. Such measurements would help assess the utility of corals as quantitative versus qualitative tracers of wastewater nitrogen. Laboratory studies could be particularly useful in this regard.

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